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RAPID COMMUNICATION

Differential cytology of bronchoalveolar lavage fluid in normal children

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ABSTRACT: Bronchoalveolar lavage (BAL) is increasingly used in the assessment of pulmonary diseases in children. However, reference values for cellular and non-cellular constituents of BAL fluid in children are lacking.

We have studied the differential cytology of BAL fluid in 48 children aged 3–16 years (mean age±SD 7.9±3.5 yrs) undergoing elective surgery for nonpulmonary illnesses. A flexible bronchoscope (Pentax 3.5 or 4.9 mm) was wedged in the middle lobe or one of its segments. BAL was performed with 3×1 ml·kg^{-1} body weight of normal saline warmed to body temperature. The first sample was studied separately; subsequent samples were pooled for analysis.

The mean recovery was 58±15%. Significantly more granulocytes and less lymphocytes were found in the first, as compared to the pooled, sample. Total cell counts were highly variable and ranged from 0.5–57.1 × 10^4·ml^{-1}. Macrophages were the predominant cell type, with a mean percentage of 81.2±12.7%. The relative proportion of lymphocytes was higher than that reported in most studies of adult volunteers (16.1±2.4%). No age dependency was observed for either cell type. The mean percentage of granulocytes was 2.5±3.3%. Absolute granulocyte counts were significantly higher in children under 8 yrs of age.

This study provides the first reference data on BAL differential cytology in children without pulmonary disease and will be the basis for future investigations of BAL in paediatric lung diseases.

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Bronchoalveolar lavage (BAL) is a valuable research tool for the assessment of the lower respiratory tract in adult respiratory medicine [1, 2]. It is also used clinically to diagnose and assess treatment response in various pulmonary diseases [3, 4]. With the invention of smaller flexible bronchoscopes in recent years, a growing number of studies have investigated bronchoalveolar lavage constituents in children [5–14]. Most of these studies have focused on the retrieval of infectious organisms in the immunocompromised host.

Only limited information exists about the cellular constituents of the bronchoalveolar surface in the paediatric age group. Cells in BAL fluid (BALF) have been studied in childhood asthma, as well as in children with human immunodeficiency virus (HIV)-related pulmonary disease [6, 15]. However, the interpretation of cellular profiles in children with lung disease has been hampered by the lack of a control population. Normal values in the paediatric age group are difficult to obtain, since the investigation of healthy children as volunteers is not feasible for ethical reasons. In order to assess the cellular components of BALF in children without pulmonary disease, we have studied children undergoing elective surgery under general anaesthesia for nonpulmonary illnesses.

Material and methods

The study population consisted of 50 children aged 3–16 yrs. Two children were excluded from the analysis because of signs of airway inflammation on bronchoscopy. The mean age of the remaining 48 children was 7.9±3.5 yrs (mean±SD). All children were undergoing elective surgery for nonpulmonary illnesses (table 1).

Table 1. – Diagnosis and operative procedure of children undergoing BAL

Diagnosis	Operative procedure	n
Hypertrophy of tonsils	Tonsillectomy	10
Hypertrophy of adenoids	Adenectomy	11
Retentio testis	Orchidopexy	7
Hypospadia	Correction	6
Vesicoureteral reflux	Ureteral reimplantation	5
Deviation of the nasal septum	Correction	2
Epispadia	Correction	2
Conductive deafness	Tympanoplasty	2
Parotic cyst	Exstirpation	1
Cervical cyst	Exstirpation	1
Urethra duplex	Correction	1

BAL: bronchoalveolar lavage.

Children with chronic respiratory symptoms, an upper respiratory tract infection in the preceding 3 months, a lower respiratory tract infection in the preceding 2 yrs, a history of hyperreactive airway disease or other atopic symptoms (allergic rhinitis, atopic dermatitis) were excluded from this study. None of the children was receiving any medications at the time of investigation. The study was approved by the Ethics Committee of our institution. Written informed consent was obtained from both parents. A questionnaire was given to all parents to obtain information about their smoking habits both at the time of study and during pregnancy. The questionnaire also included questions related to hyperreactive airway disease in the child and first degree relatives.

BAL was performed under general anaesthesia and tracheal intubation. All procedures were performed by one investigator (FR). Children received flunitrazepam (0.04 –0.05 mg·kg^{-1} body weight) orally, 1 h prior to the procedure. In children under 6 yrs, anaesthesia was initiated *via* inhalation of a nitrous oxide-oxygen mixture with 2.5 volume (vol) % halothane. Subsequently, a venous access was inserted and atropine (0.01 mg·kg^{-1} body weight), vecuronium (0.05 mg·kg^{-1} body weight) and alfentanil (0.05 mg·kg^{-1} body weight) were given intravenously. The halothane concentration was then reduced to 1.5 vol %. In children older than 6 yrs, a venous access was inserted first and methohexitone, 1–1.5 mg·kg^{-1} body weight, was given followed by atropine, vecuronium and alfentanil, as described above. After intubation, the oxygen concentration was raised to 100% with 1.5 vol % halothane for the bronchoscopy procedure.

A flexible bronchoscope with an external diameter of 3.5 or 4.9 mm (Pentax, Hamburg, Germany) was passed through the endotracheal tube and wedged in the right middle lobe or one of its segments. Airway inflammation was assessed with the bronchitis index proposed by THOMPSON *et al.* [16]. Only children with no signs of airway inflammation (bronchitis index=0) were considered normal and included in the analysis. BAL was performed with normal saline, warmed to body temperature (37°C). Three × 1 ml·kg^{-1} body weight were instilled in all children. In subjects weighing less than 20 kg, BAL was performed in three equal portions. In children weighing more than 20 kg, 20 ml portions were instilled, up to a total volume of 3 × 1 ml·kg^{-1} body weight. The first sample was studied separately; subsequent samples were pooled for analysis.

BAL fluid was filtered through sterile gauze and centrifuged at 500×g for 10 mins. The cell suspension was washed three times in Eagle's minimal essential medium (MEM) containing 0.2% bovine serum albumin and 0.1% ethylenediamine tetra-acetic acid (EDTA) and resuspended in MEM. Cell viability was assessed by trypan blue exclusion test. Differential cell counts were obtained from smears stained with May-Grünwald-Giemsa. At least 600 cells were counted in each subject.

Statistical analysis

All data were tested for normal distribution with the Kolmogorov-Smirnov test. Cell counts were expressed both as cells·ml^{-1} BAL fluid recovered and as percentage of total cells. Results were expressed as mean± standard deviation (SD). The median is also reported for all data. Linear regression analysis was performed to assess age dependency of the variables. In addition, two different age groups (3–8 yrs and 9–16 yrs) were generated to assess age-related differences with the Wilcoxon test. Differences between the first and the pooled sample were assessed with the Mann-Whitney U-test. A p-value of less than 0.05 was considered as statistically significant.

Results

No side-effects of the BAL were noted, except for a low grade fever up to 38°C in four subjects that resolved spontaneously within 24 h. One subject was assessed twice because of an upper respiratory tract infection at the time of the first bronchoscopy that was unknown by the person performing the procedure. His first BAL showed relative and absolute lymphocytosis that was not present on the second occasion 6 months later.

The mean recovery was 58±15% (mean±SD). As expected, the recovery of the first sample was lower as compared to the pooled sample (36±13 *vs* 65±18%). Vitality assessed by trypan blue exclusion was 78±18% (mean±SD).

A separate analysis of the differential cytology of the first sample could be performed in 39 children. Compared to the pooled sample, the first portion of the BAL contained significantly more granulocytes and less lymphocytes (fig. 1). No differences were observed in the relative proportion of macrophages.

The results of the differential cytology of the pooled sample is shown in table 2. There was considerable variability both in the absolute and relative cell counts of all measured parameters. Both absolute and relative cell counts did not follow a normal distribution. No age-related differences were seen in the percentage of macrophages and lymphocytes (figs. 2 and 3). The proportion of granulocytes was more variable in children under 8 yrs of age (fig. 4). When expressed in cells·ml^{-1} of recovered BAL fluid, total cell counts tended to be

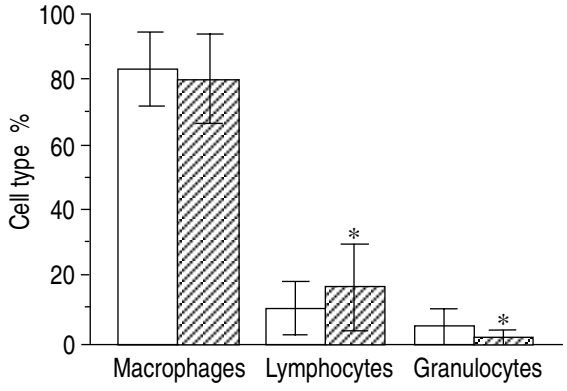

Fig. 1. – Comparison of differential cytology of the first (□) *versus* the pooled (▨) bronchoalveolar lavage samples. Mean values ±SD are displayed. The first sample contained significantly more granulocytes and less lymphocytes (*: p<0.001, Mann-Whitney U-test).

Table 2. – Differential cytology of the pooled BAL samples

Cell type	Mean	SD	Median	Min	Max
Macrophages %	81.2	12.7	84	34.6	94
Lymphocytes %	16.2	12.4	12.5	2	61
Granulocytes %	2.5	3.3	1.6	0.2	19
Neutrophils	1.9	2.9	0.9	0	17
Eosinophils	0.4	0.6	0.2	0	3.6
Basophils	0.3	0.5	0.1	0	2.8
Cells·kg^{-1} ×10^4	14.6	13.3	10.7	1.5	66.8
Cells·ml^{-1} ×10^4	10.3	11.1	7.3	0.5	57.1
Macrophages·ml^{-1}	8.3	8.6	6.4	0.4	48
Lymphocytes·ml^{-1}	1.8	3.4	1	0.1	23
Granulocytes·ml^{-1}	0.3	0.4	0.1	0	2.2

BAL: bronchoalveolar lavage.

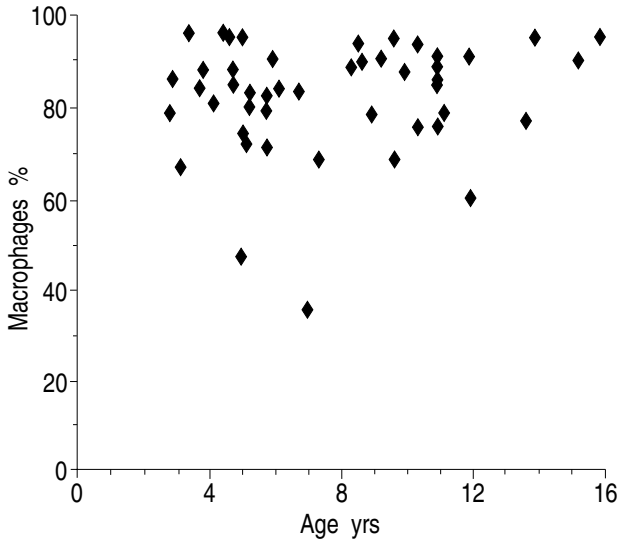

Fig. 2. – Relative proportions of macrophages in the pooled samples as a function of age. Each diamond represents one individual.

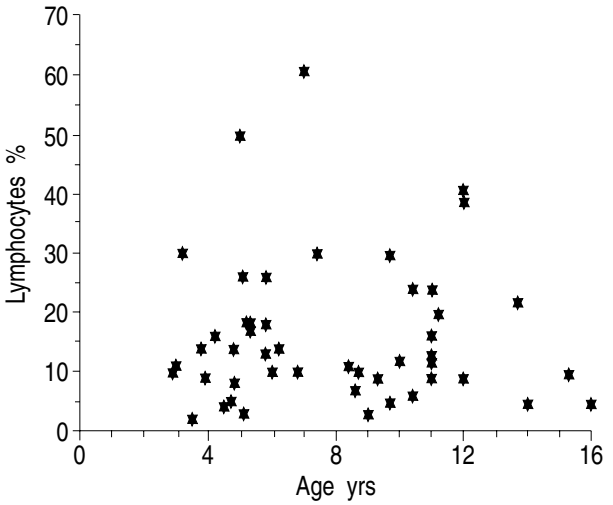

Fig. 3. – Relative proportions of lymphocytes in the pooled samples as a function of age. Each asterix represents one individual.

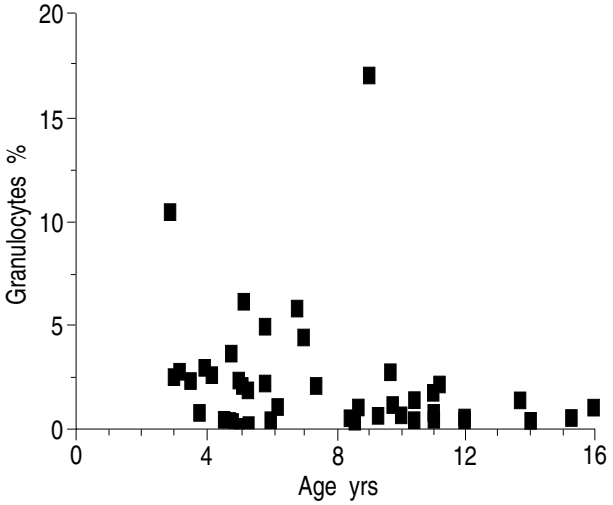

Fig. 4. – Relative proportions of granulocytes in the pooled samples as a function of age. Each square represents one individual.

Table 3. – Absolute cell counts (cells×10^4·ml^{-1}) of recovered BAL fluid (pooled samples) according to age

Cell type	Age yrs	Mean	SD	n	p-value
Total cells·ml^{-1}	3–8	11.7	11.3	25	
	9–16	6.6	5.4	23	0.05
Macrophages·ml^{-1}	3–8	10.1	9.7	25	
	9–16	6.2	6.7	23	0.12
Lymphocytes·ml^{-1}	3–8	2.5	4.6	25	
	9–16	1.1	1.2	23	0.16
Granulocytes·ml^{-1}	3–8	0.39	0.54	25	
	9–16	0.1	0.11	23	0.02

BAL: bronchoalveolar lavage.

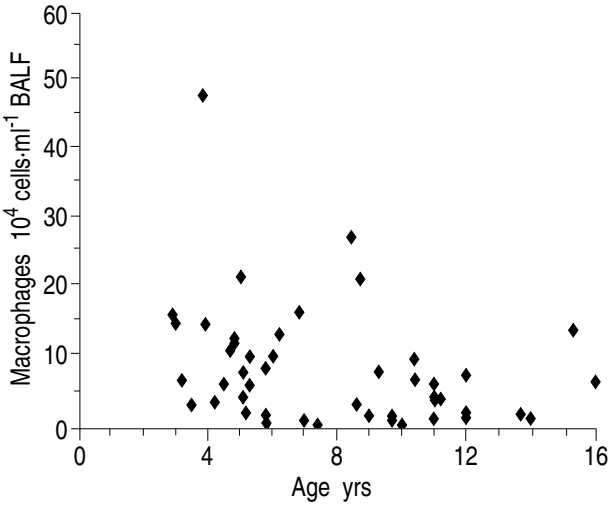

Fig. 5. – Macrophages·ml^{-1} of pooled BALF as a function of age. Each diamond represents one subject. BALF: bronchoalveolar lavage

higher in younger children (table 3). Macrophages·ml^{-1} and lymphocytes·ml^{-1} were independent of age (figs 5 and 6), whilst granulocyte counts tended to be increased in younger children (fig. 7). This was reflected by significantly more granulocytes·ml^{-1} of recovered BAL fluid in children younger than 8 yrs (table 3).

The comparison of differential cytology of children undergoing urological operations as compared to upper

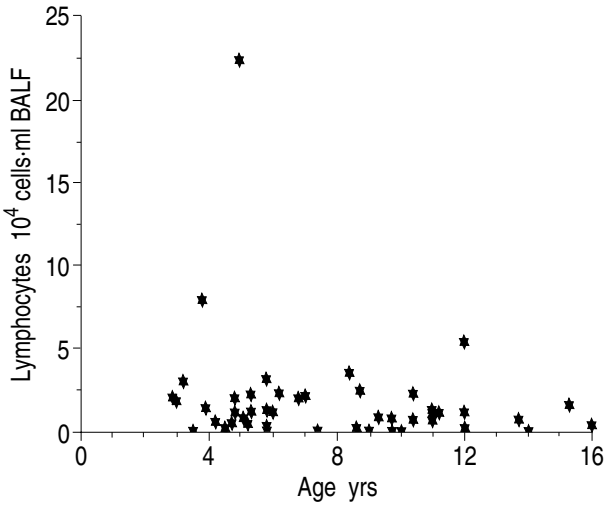

Fig. 6. – Lymphocytes·ml^{-1} of pooled BALF as a function of age. Each asterix represents one individual. BALF: bronchoalveolar lavage fluid.

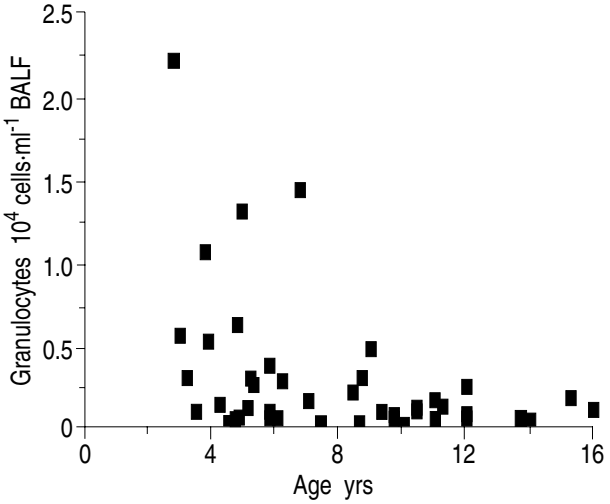

Fig. 7. – Granulocytes·ml^{-1} of pooled BALF as a function of age. Each square represents one individual. BALF: bronchoalveolar lavage fluid.

Table 4. – Differential cell counts in children with upper airway *versus* urological operations

	Upper airway operation	Urological operation	p-value*
n	27	21	
Age yr	8.1±3.9	7.7±3	0.69
Recovery %	57±16	59.0±14	0.67
Macrophages %	80.2±13.8	82.7±11.2	0.51
Lymphocytes %	17.7±13.3	14.3±11	0.25
Granulocytes %	2.2±2.2	3.0±4.4	0.96
Cells·ml^{-1} ×10^4	9.6±9.2	8.9±9.6	0.48
Macrophages·ml^{-1}	8.5±9.1	8.0±8.1	0.60
Lymphocytes·ml^{-1}	1.7±1.7	2.0±4.9	0.17
Granulocytes·ml^{-1}	0.2±0.3	0.3±0.5	0.15

Results are expressed as mean±SD. *Wilcoxon test.

airway operations is shown in table 4. There were no differences in age, recovery, relative and absolute cell counts in the two populations.

The questionnaire was returned in 39 cases. Fourteen children were exposed to more than 10 cigarettes·day^{-1}; three parents smoked less than 10 cigarettes·day^{-1}; 22 parents never smoked. No differences could be observed between the children of smokers and nonsmokers in absolute cell counts, cell counts·ml^{-1}, or differential cytology.

Discussion

Studies in adults have described alterations in the cellular composition of BALF in various pulmonary diseases [3, 4]. Whilst the spectrum of lung disease in children differs from that of adults, BAL can also be expected to play an important role in the assessment of childhood pulmonary disorders. Defining epithelial lining fluid contents in normal children is an essential prerequisite for the interpretation of changes observed in lung diseases. This study was the first step to define the cellularity of BALF in children without pulmonary disorders.

We observed a wide range in the total cell counts of the BALF samples of our children. This is in concordance with the marked intra- and interindividual variability previously demonstrated in adults, making the analysis of total cell counts unsuitable for differentiating between normals and subjects with lung disease [17, 18]. Interestingly, the total cell counts in our population tended to be higher in younger children. One factor that is known to increase total cell counts is smoking [19–21]. We have assessed the smoking history of the parents by questionnaire, and were unable to relate passive smoking to the absolute cell count of the recovered BALF. However, questionnaires are not highly reliable in determining smoking exposure [22]. While we found no relationship between passive smoking and BALF cellularity, a more detailed study using urine cotinine levels may be warranted to address this question.

A considerable variability was also observed in the relative proportion of lymphocytes in this study. Investigations of normal nonsmoking adult subjects have found mean lymphocyte counts between 4–18% of the recovered cells [17–20, 22–30], with most of the studies reporting mean values of less than 10%. The mean fraction of lymphocytes in our study was 16.2±12.4% with a median of 12.5%, and therefore higher than in most studies of normal adults. As has been reported in larger series of adult subjects, cells were not normally distributed, with individual subjects exceeding lymphocyte counts of 30%. With the exception of three children, total lymphocytes counts·ml^{-1} were below 50,000, which is well within the range reported in normal nonsmoking adult volunteers, whereas in 12 of our 48 children, the lymphocyte percentage exceeded 20%, and, thus, the upper limits of normal of most adult studies.

We are unable to explain the variability in the differential and total cell counts observed in this study. As has been demonstrated in adults, some individuals may have subclinical alveolitis without clinical, radiological or functional abnormalities [31]. This phenomenon can also be expected to occur in children, and may partially account for the high variability in lymphocyte cell counts.

An alternative explanation would be that prior respiratory viral infections may have caused changes in the cellularity on the bronchoalveolar surface. Even though all children were free of respiratory tract infections in the preceding 3 months, little information is available on the duration of changes in BAL cells that are induced by respiratory viral infections. Acute respiratory viral infections occur with a high frequency, especially in younger children [32]. It appears likely that this increased incidence in infections could influence the variability of lymphocyte counts in the paediatric age group. Whether the higher lymphocyte counts that we observed in individual children were a transient phenomenon or persisted for longer periods could only be clarified if subjects were followed longitudinally, which was not feasible in our study population.

Granulocyte counts were higher in younger children. Several mechanisms may contribute to this phenomenon. As has been described in studies in adults [33, 34], we found a higher proportion of granulocytes in the first as compared to the pooled sample. Anatomical studies indicate that lung growth consists of rapid proliferation of alveoli in the first 6 yrs of life, with most of the changes occurring up to the age of 2 yrs [35]. Airway growth, in contrast, is relatively constant in childhood [36]. One would, therefore, expect that the contribution of alveolar lining fluid to the BAL sample is smaller in younger children. This would result in the presence of relatively more bronchial lining fluid in a BAL of younger children, as compared to older subjects. A change in the contribution of the amount of bronchial lining fluid sampled may also be explained by technical differences. All children up to the age of 10 yrs were studied with the same bronchoscope (Pentax 3.5 mm). The bronchoscope was, thus, wedged in more proximal airways in younger subjects, resulting in a different relationship of fluid sampled from the airways and alveoli between younger and older children. Both the anatomical and technical considerations would support the hypothesis that the higher granulocyte counts in younger children are caused by a greater contribution from the airways to the pooled BAL sample.

In summary, we have studied cellular constituents of BALF in children without pulmonary disease. Significantly more granulocytes and less lymphocytes were present in the first as compared to the pooled sample. Macrophages were the predominant cell type, followed by lymphocytes. Absolute granulocyte counts were higher in younger children, probably due to a larger contribution of bronchial lining fluid to the BAL sample. These studies provide a first insight into BALF cellularity in the paediatric age group, and will be the basis for further studies in children with lung disease.

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